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## Datasheet for ABIN414374

## **Fibronectin ELISA Kit**





#### Overview

Quantity:	96 tests
Target:	Fibronectin
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.156 ng/mL - 10 ng/mL
Minimum Detection Limit:	0.156 ng/mL
Application:	ELISA
Product Details	
Purpose:	The kit is a high sensitive sandwich enzyme immunoassay for in vitro quantitative
	measurement of fibronectin in human serum, plasma, tissue homogenates, cell lysates, cell
	culture supernates.
	We offer <b>validation data</b> (WB) <b>for the kit components</b> . So you can be sure to order a reliable ELISA kit product composed of high quality reagents.
Sample Type:	Cell Culture Supernatant, Cell Lysate, Plasma, Serum, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of High Sensitive
	Fibronectin (FN).
	No significant cross-reactivity or interference between High Sensitive Fibronectin (FN) and

#### **Product Details**

	analogues was observed.
Cross-Reactivity (Details):	No significant cross-reactivity or interference between High Sensitive Fibronectin (FN) and analogues was observed.
Sensitivity:	0.053 ng/mL
Grade:	High Sensitivity
Components:	<ul> <li>Pre-coated, ready to use 96-well strip plate, flat buttom</li> <li>Plate sealer for 96 wells</li> <li>Reference Standard</li> <li>Standard Diluent</li> <li>Detection Reagent A</li> <li>Detection Reagent B</li> <li>Assay Diluent A</li> <li>Assay Diluent B</li> <li>Reagent Diluent (if Detection Reagent is lyophilized)</li> <li>TMB Substrate</li> <li>Stop Solution</li> <li>Wash Buffer (30 x concentrate)</li> <li>Instruction manual</li> </ul>

## **Target Details**

Target:	Fibronectin
Abstract:	Fibronectin Products
UniProt:	P02751

#### **Application Details**

#### Application Notes:

- Limited by the current condition and scientific technology, we cannot completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit.
- The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.
- Kits from different batches may be a little different in detection range, sensitivity and color developing time.
- Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
- · Protect all reagents from strong light during storage and incubation. All the bottle caps of

- reagents should be covered tightly to prevent the evaporation and contamination of microorganism.
- There may be some foggy substance in the wells when the plate is opened at the first time. It
  will not have any effect on the final assay results. Do not remove microtiter plate from the
  storage bag until needed.
- Wrong operations during the reagents preparation and loading, as well as incorrect
  parameter setting for the plate reader may lead to incorrect results. A microplate plate reader
  with a bandwidth of 10nm or less and an optical density range of 0-3 0.D. or greater at 450 ±
  10nm wavelength is acceptable for use in absorbance measurement. Please read the
  instruction carefully and adjust the instrument prior to the experiment.
- Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended.
- Each kit has been strictly passed Q.C test. However, results from end users might be
  inconsistent with our in-house data due to some unexpected transportation conditions or
  different lab equipments. Intra-assay variance among kits from different batches might arise
  from above factors, too.
- Kits from different manufacturers for the same item might produce different results, since
  we have not compared our products with other manufacturers.

#### Comment:

Information on standard material:

The standard might be recombinant protein or natural protein, that will depend on the specific kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin 300 in the standard as preservative.

Information on reagents:

The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them.

Information on antibodies:

The provided antibodies and their host vary in different kits.

Sample Volume: 100 μL

Assay Time: 3 h

Plate: Pre-coated

Protocol: The test principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to High Sensitive Fibronectin (FN). Standards or samples are then added to the appropriate microtiter plate wells with a

biotin-conjugated antibody specific to High Sensitive Fibronectin (FN). Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain High Sensitive Fibronectin (FN), biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of High Sensitive Fibronectin (FN) in the samples is then determined by comparing the O.D. of the samples to the standard curve.

#### Reagent Preparation:

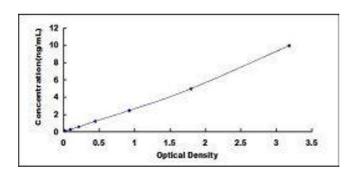
- 1. Bring all kit components and samples to room temperature (18-25 °C) before use. If the kit is not used up all at once, remove only the strips and reagents for the current experiment and leave the remaining strips and reagents in the desired condition.
- 2. Standard Reconstitute the Standard with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 20ng/mL. First dilute the stock solution to 10ng/mL and the diluted standard serves as the highest standard (10ng/mL). Then prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, 0.312ng/mL, 0.156ng/mL, and the last tube with Standard Diluent is the blank as 0ng/mL.
- 3. **Detection Reagent A** and **Detection Reagent B** Spin or centrifuge the stock of Detection Reagent A and B briefly before use. Dilute to working concentration (1:100) with Assay Diluent A or B, respectively.
- 4. **Wash Solution** Dilute 20 mL of Wash Solution Concentrate (30x) with 580 mL of deionized or distilled water to make 600 mL of Wash Solution (1x).
- 5. **TMB Substrate** Aspirate the required amount of solution with sterile tip and do not return the residual solution back into the vial.

#### Note:

- 1. Serial dilution directly in the wells is not recommended.
- 2. Prepare standard within 15 minutes before assay. Do not dissolve the reagents directly at 37 °C.
- 3. Detection Reagent A and B are sticky solutions, so pipette them slowly to reduce volume errors.
- 4. Reconstitute Standard or working solutions of Detection Reagent A and B carefully according to instructions, avoiding foaming and mixing gently until crystals are completely dissolved. To minimize inaccuracy caused by pipetting, use small volumes and ensure pipettes are calibrated. It is recommended to aspirate more than 10 µL for one-time pipetting.
- 5. The reconstituted Standard, Detection Reagent A and B can only be used once.
- 6. When crystals have formed in the Wash Solution concentrate (30x), warm it to room temperature and mix gently until the crystals are completely dissolved.
- 7. Contaminated water or preparation containers affect the detection result.

# **Application Details**

Assay Precision:	Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level
	High Sensitive Fibronectin (FN) were tested 20 times on one plate, respectively.
	Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level
	High Sensitive Fibronectin (FN) were tested on 3 different plates, 8 replicates in each plate.
	CV(%) = SD/meanX100
	Intra-Assay: CV<10%
	Inter-Assay: CV<12%
Restrictions:	For Research Use only
Handling	
Precaution of Use:	The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and
	clothing protection when using this material.
Handling Advice:	The stability of kit is determined by the loss rate of activity. The loss rate of this kit is less than
	5 % within the expiration date under appropriate storage condition.
	To minimize extra influence on the performance, operation procedures and lab conditions,
	especially room temperature, air humidity, incubator temperature should be strictly controlled. I
	is also strongly suggested that the whole assay is performed by the same operator from the
	beginning to the end.
Storage:	4 °C
Storage Comment:	For unopened kit: All the reagents should be kept according to the labels on vials. The
	Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be
	stored at -20°C upon receipt while the others should be at 4°C.
	For opened kit: When the kit is opened, the remaining reagents still need to be stored
	according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal.
	Note: It is highly recommended to use the remaining reagents within 1 month provided this is
	within the expiration date of the kit.
	• For ELISA kit, 1 day storage at 37°C can be considered as 2 months at 4°C, which means 3
	days at 37°C equaling 6 months at 4°C.
Expiry Date:	6 months



### **ELISA**

Image 1.